



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

NAZARENKO *et al.*

Appl. No.: 09/599,594

Filed: June 22, 2000

For: **Primers and Methods for the
Detection and Discrimination of
Nucleic Acids**

Confirmation No.: 8750

Art Unit: 1637

Examiner: Fredman, J.N.

Atty. Docket: 0942.4980002/RWE/FRC

Brief on Appeal Under 37 C.F.R. § 41.37

Mail Stop Appeal Brief - Patents

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

A Notice of Appeal from the final objection of claims 21, 22, and 76-79 and the final rejection of claims 11, 12, 14, 15, 17-20, 59 and 63-67 was filed on July 3, 2006. Appellants hereby file this Appeal Brief, together with the required brief filing fee.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Table of Contents

Table of Contents.....	i
I. Real Party In Interest	1
II. Related Appeals and Interferences	2
III. Status of Claims.....	3
IV. Status of Amendments.....	4
V. Summary of Claimed Subject Matter	5
VI. Grounds of Rejection to be Reviewed on Appeal	7
VII. Argument	8
A. 35 U.S.C. § 102(e)	
B. 35 U.S.C. § 103(a)	
1. The Legal Standard.....	4
2. The Cited References.....	5
a. The Horn Reference.....	10
b. The Tyagi Reference	11
3. The Examiner's Position	11
4. The Appellants' Position.....	12
5. Conclusion.....	10
VIII. Claims Appendix	17
IX. Evidence Appendix.....	21

X. Related Proceedings Appendix.....	22
--------------------------------------	----



NAZARENKO *et al.*
Appl. No. 09/599,594

I. Real Party In Interest

The real party in interest in this Appeal is Invitrogen Corporation.

II. Related Appeals and Interferences

No other prior or pending appeals, interferences or judicial proceedings are known to the Appellants or the Appellants' legal representatives which may be related to, or directly affect, or be directly affected by, or have a bearing on the Board's decision in the pending Appeal.

III. Status of Claims

Claims 11, 12, 14, 15, 17-19, 59, 63-67, 78 and 79 are pending in the application.

Claims 1-10, 13, 16, 20-22*, 23-58, 60-62, 68-75, 76-77*, and 80-85 have been canceled.

Claims 11-12, 14-15, 17-19, 59 and 63-67 are rejected.

Claims 78-79 are objected to.

* Claims 20-22, 76 and 77 are canceled in an Amendment Under 37 C.F.R. § 41.33(b), filed concurrently herewith.

IV. Status of Amendments

In an Amendment Under 37 C.F.R. § 41.33(b), filed concurrently herewith, Applicants have requested that claims 20-22, 76 and 77 be canceled and that claims 17 and 63-67 be amended to change their dependencies so that these claims do not depend from a canceled claim.

V. Summary of Claimed Subject Matter

Claims 11, 12 and 18 are the independent claims involved in this Appeal. The invention defined by claim 11 relates generally to a method for quantifying or detecting nucleic acid molecules during nucleic acid synthesis. The method of claim 11 involves mixing a target nucleic acid with a fluorescently-labeled oligonucleotide. The oligonucleotide has a single type of fluorescent label with the same chemical structure, and undergoes a detectable change in fluorescence upon hybridization to the target nucleic acid. The mixture containing the target nucleic acid and the fluorescently-labeled oligonucleotide is incubated under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of the target nucleic acid whereby the labeled oligonucleotide is incorporated into the synthesized nucleic acid. Support for claim 11 can be found throughout the Specification, for example, at page 16, lines 4-6 and 13-30; page 17, lines 1-5 and 17-20; page 45, lines 16-20; page 46, lines 7-15; and Example 3.

Claim 12 relates generally to a method for quantitation or detection of nucleic acid molecules during nucleic acid amplification. The method of claim 12 involves mixing a target nucleic acid with a fluorescently-labeled oligonucleotide. The oligonucleotide has a single type of fluorescent label with the same chemical structure, and undergoes a detectable change in fluorescence upon hybridization to the target nucleic acid. The mixture containing the target nucleic acid and the fluorescently-labeled oligonucleotide is incubated under conditions sufficient to amplify a nucleic acid molecule complementary to all or a portion of the target nucleic acid whereby the labeled

oligonucleotide is incorporated into the amplified nucleic acid. Support for claim 12 can be found throughout the Specification, for example, at page 16, lines 4-6 and 25-30; page 17, lines 1-5 and 17-20; in Figure 1; page 45, lines 16-20; and Example 3.

Claim 18 relates generally to a method for amplifying a double stranded nucleic acid. The method of claim 18 involves the use of primers that have a single type of fluorescent label with the same chemical structure and undergo a detectable change in fluorescence upon hybridization to a complementary nucleic acid. The primers are hybridized to opposite strands of the double stranded target nucleic acid in the presence of a polymerase under conditions sufficient to extend and incorporate the primers into a newly synthesized complementary nucleic acid. Amplification of the double stranded target nucleic acid occurs with repeated rounds of denaturation, hybridization and primer extension. Support for claim 18 can be found throughout the Specification, for example, at page 16, lines 4-6; page 17, lines 17-20; page 18, lines 1-18; page 20, lines 11-27; in Figure 1; and Example 2.

VI. *Grounds of Rejection to be Reviewed on Appeal*

A. *35 U.S.C. § 102(e)*

Claim 20 stands rejected under 35 U.S.C. § 102(e) as being anticipated by Horn *et al* (U.S. Patent No. 6,465,175) (Exhibit 1).

B. *35 U.S.C. § 103(a)*

Claims 11-12, 14-15, 17-20, 59 and 63-67 stand rejected under 35 U.S.C. § 103(a), as being unpatentable over Horn *et al* (US Patent No. 6, 465,175) in view of Tyagi *et al* (US Patent No. 6,037,130) (Exhibit 2).

VII. Argument

A. 35 U.S.C. § 102(e)

Claim 20

By way of the amendment filed concurrently, herewith under 37 CFR § 41.33(b), claim 20 has been cancelled. Thus, the rejection of this claim is rendered moot.

B. 35 U.S.C. § 103(a)

Claim 20

By way of the amendment filed concurrently, herewith under 37 CFR § 41.33(b), claim 20 has been cancelled. Thus, the rejection of this claim is rendered moot.

Claims 11-12, 14-15, 17-19, 59 and 63-67

Appellants respectfully traverse this rejection with respect to claims 11-12, 14-15, 17-19, 59 and 63-67.

1. Legal Standard

In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. *See In re Piasecki*, 745 F.2d 1468, 1471-73, 223 USPQ 785, 788 (Fed. Cir. 1984). To meet this burden, the Examiner must satisfy three requirements. First, all of the claim limitations must be taught or suggested by the prior art. *See In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974); see also *In re Glaug*, 283 F.3d 1335, 1341-42, 62 USPO2d 1151,

1154 (Fed. Cir. 2002); *In re Rijckaert*, 9 F.3d 1531, 1533, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine references. *See In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998). Third, there must be a reasonable expectation of success. *See In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir.1986). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in Applicants' disclosure. *See In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Evidence of a suggestion, teaching, or motivation to combine prior art references may flow, *inter alia*, from the references themselves, the knowledge of one of ordinary skill in the art, or from the nature of the problem to be solved. *See In re Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999). Although a reference need not expressly teach that the disclosure contained therein should be combined with another, the showing of combinability, in whatever form, must nevertheless be "clear and particular." *Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617. "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence.'" *Id.* At 999, 50 USPQ2d at 1617, *see also In re Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1318 (Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.") Absent a showing

of such motivation and suggestion, *prima facie* obviousness is not established. See *In re Fine*, 5 USPQ2d at 1598.

The teachings of prior art references must be considered in their entirety, *i.e.* as a whole, including portions that teach away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984). The Court of Appeals for the Federal Circuit has instructed that “references that teach away cannot serve to create a *prima facie* case of obviousness” (*In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994)), and that an “applicant may rebut a *prima facie* case of obviousness by showing that the prior art teaches away from the claimed invention in any material respect” (*In re Geisler*, 116 F.3d 1465, 1469 (Fed. Cir. 1997)).

2. The Cited References

The Horn Reference

The Horn reference discloses a probe-based method for detecting target nucleic acids. Horn’s method involves mixing target nucleic acid molecules with an oligonucleotide probe having a single fluorescent label (*i.e.*, a single-labeled probe). The premise behind Horn’s method is that when a single-labeled probe is in non-hybridized form it provides a detectable fluorescent signal, but when the probe hybridizes to a complementary target nucleic acid it undergoes a spontaneous conformational change and does not fluoresce. See Horn column 3, lines 6-20 and column 17, lines 65-67.

The Tyagi Reference

The Tyagi reference discloses a wavelength-shifting molecular beacon-based method for detecting target nucleic acids. Tyagi's wavelength-shifting molecular beacon-based detection method involves mixing target nucleic acids with a multiple-labeled primer or probe having a pair of fluorophores (*i.e.*, a harvester and an emitter moiety) positioned near one end of the primer/probe. The multiple-labeled primers or probes used in Tyagi's wavelength-shifting molecular beacon-based detection method also include a fluorescence-quenching moiety, which itself may be a fluorophore and is located at a end of the primer/probe opposite the emitter and harvester. See Tyagi column 2, lines 45-65 and column 5, lines 36-38. The premise behind Tyagi's wavelength-shifting molecular beacon-based detection method is that upon hybridization to a target nucleic acid the multiple-labeled primer or probe undergoes a conformation change that eliminates quenching and allows detection of fluorescence by the emitter and/or harvester. More specifically, the pair or fluorophores on the primer or probe are allowed to interact by fluorescence resonance energy transfer (FRET) when the quencher label is separated from them by hybridization to a target nucleic acid. See Tyagi, column 8, lines 13-60.

3. *The Examiner's Position*

The Examiner argues that the method of claims 11-12, 14-15, 17-19, 59 and 63-67 are obvious over Horn in view of Tyagi. The Examiner's position is that the skilled artisan would have been motivated to arrive at the Appellant's single label primer-based detection method by modifying the multiple-labeled primers used in Tyagi's wavelength-

shifting molecular beacon-based detection method to use only a single label.

Specifically, the Examiner states that:

[M]otivation to use the modify [*sic*] the Tyagi primers to use a single label is present when Horn notes “Accordingly, single label quenching molecular beacons can be used for the detection of nucleic acids in homogeneous assays and in living cells, as well as for real time monitoring of assays in which nucleic acids are being synthesized, e.g. polymerase chain reactions.”

See Office Action dated January 4, 2006 at page 6.

4. *The Appellants' Position*

Claims 11-12, 14-15, 17-19, 59 and 63-67 are not obvious in view of the Horn and Tyagi references.

The claimed methods involve the detection of single-labeled oligonucleotides incorporated into synthesized nucleic acids. The claimed methods offer several advantages over the prior art including, for example:

- a.) allowing detection of the amplification or synthesis product directly, by incorporating the labeled oligonucleotide into the product;
- b.) not requiring labeling of oligonucleotides with two different compounds (like FRET-based methods).

See Specification at page 10, lines 16-19.

Neither the Horn nor the Tyagi reference disclose the use of single-labeled primers that are incorporated into synthesized nucleic acids. Detection using the probe-

based methods described by Horn is accomplished by nucleic acid hybridization, which does not involve extension and incorporation of a primer in an amplification product to be detected. Thus, as the Examiner acknowledges (see Office Action dated January 4, 2006, page 6), Horn fails to teach incorporation of a labeled oligonucleotide into a synthesized nucleic acid product.

The Examiner relies on the Tyagi reference to cure this deficiency. Specifically, the Examiner states that Tyagi “teaches the use of fluorescently labeled molecular beacon primers being incorporated into the PCR product.” See Office Action dated January 4, 2006 at page 6. The Examiner further states that “an ordinary practitioner is motivated to substitute the single label quenching beacons of Horn in the method of Tyagi so that a separate quenching dye is not necessary.” See Office Action dated January 4, 2006 at page 6. The Examiner states that this motivation is “present when Horn notes ‘Accordingly, single label quenching molecular beacons can be used for the detection of nucleic acids in homogenous assays and in living cells, as well as for real time monitoring of assays in which nucleic acids are being synthesized, *e.g.*, polymerase chain reactions (see column 18 lines 43-47).’” See Office Action dated January 4, 2006 at page 6.

Tyagi expressly teaches that multiple fluorophores (*i.e.*, an emitter and a harvester) are required for his wavelength-shifting molecular beacon primers/probes to function according to his invention. Specifically, Tyagi states that the methods of his invention “*require* that the *two* fluorophores be separated by an appropriate distance and that the emission spectrum of the harvester moiety significantly overlaps the absorption spectrum of the emitter moiety.” See column 7, lines 46-50 (emphasis added). Tyagi

further emphasizes the importance of using multiple fluorescent labels stating that “the transfer of energy from the harvester fluorophore to the emitter fluorophore is governed by the rules of fluorescence resonance energy transfer [FRET] which we use to aid in the design of probes and primers according to this invention.” See column 8, lines 49-53. Because Tyagi’s teachings are directly contrary to the presently claimed methods in this material respect (*i.e.*, teaching the use and requirement of multiple fluorescently-labeled rather than single fluorescently-labeled oligonucleotides), the present rejection of the claimed methods as *prima facie* obviousness in view of Tyagi is improper. See *In re Geisler*, 116 F.3d 1465, 1469 (Fed. Cir. 1997) (“applicant may rebut a *prima facie* case of obviousness by showing that the prior art teaches away from the claimed invention in any material respect”). In other words, because Tyagi clearly teaches away from the presently claimed invention (teaching the use and requirement of multiple-labeled rather than single-labeled oligonucleotides), Tyagi cannot serve as the basis for a proper §103 rejection, and therefore cannot cure the deficiency of the Horn reference. See *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994) (“references that teach away cannot serve to create a *prima facie* case of obviousness”).

Even if the Tyagi reference were properly available to cure the deficiency of the Horn reference (which, for the reasons set forth above, it is not), the combination of the cited references does not render the claimed methods *prima facie* obvious. This is because combining the cited references as the Examiner suggests fails to yield the claimed invention. Specifically, if Tyagi’s wavelength-shifting molecular beacon primers were modified as the Examiner suggests, (*i.e.*, “so that a separate quenching dye is not necessary”), the skilled artisan still does not arrive at the claimed invention, which

requires that an oligonucleotide be labeled with only a *single* fluorescent label. This is because Tyagi's invention is based on a primer/probe design that involves multiple labels (*i.e.*, two fluorophores, the emitter and the harvester, as well as a quencher moiety). Thus, even if a skilled artisan were to remove the quenching dye from Tyagi's wavelength-shifting molecular beacon primer, the resultant modified primer would still have two fluorophores (*i.e.*, an emitter and a harvester), and not just one label as required by the present claims.

Finally, the Examiner has provided no evidence to show that a skilled artisan would be motivated, much less have any reasonable expectation of success in arriving at the claimed methods based on the Horn and Tyagi references. The language quoted by the Examiner allegedly illustrating motivation to combine the cited references appears in an Example in the Horn reference that relates to the use of *probes*, which are not extended and incorporated into an amplification product for detection. While this section of the Horn reference does suggest the use of single-label probes for nucleic acid detection, it provides no proof demonstrating that the described methods would actually work. Certainly, there is not even a suggestion in the Horn reference to use a single-label *primer* (which is incorporated into an amplification product) for nucleic acid detection – much less any demonstration of operability of such a primer. The Examiner has offered no evidence to fill this deficiency.

Because Tyagi teaches away from the claimed methods and therefore is not available to cure the deficiency of Horn; because the combination of the cited references would not yield the claimed invention; and because no showing has been made that those skilled in the art would have been motivated (much less have a reasonable expectation of

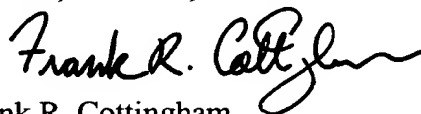
success) to practice the claimed methods by combining the cited references, *prima facie* obviousness has not been established.

5. Conclusion

In view of the forgoing discussion, Appellants respectfully submit that the subject matter defined by claims 11-12, 14-15, 17-19, 59 and 63-67 is patentable over the cited art and that the Examiner has not met the burden of establishing a *prima facie* case of obviousness. Accordingly, Appellants respectfully request that the Board reverses the Examiner's final rejection of these claims under 35 U.S.C. § 103(a) and remand this application for issue.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Frank R. Cottingham
Attorney for Applicants
Registration No. 50,437

Date: SEP. 5, 2006

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

578564_1.DOC

VIII. Claims Appendix

11. A method for the quantitation or detection of one or more target nucleic acid molecules in a sample during nucleic acid synthesis comprising:

mixing one or more a target nucleic acid molecules with one or more fluorescently labeled oligonucleotides, wherein said one or more oligonucleotides are labeled with only a single type of fluorescent label, said single type of fluorescent label having the same chemical structure, and said oligonucleotide undergoes a detectable change in fluorescence upon hybridization of said one or more oligonucleotides to said one or more target nucleic acid molecules;

incubating said mixture under conditions sufficient to synthesize one or more nucleic acid molecules complementary to all or a portion of said one or more target nucleic acid molecules, said one or more synthesized nucleic acid molecules comprising said one or more oligonucleotides; and

detecting the presence or absence or quantifying the amount of said one or more synthesized nucleic acid molecules by measuring said fluorescent label.

12. A method for quantitation or detection of one or more target nucleic acid molecules in a sample during nucleic acid amplification comprising:

mixing one or more target nucleic acid molecules with one or more fluorescently labeled oligonucleotides under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said one or more target nucleic acid molecules, said one or more amplified nucleic acid molecules comprising said one or more oligonucleotides, wherein said one or more oligonucleotides are labeled with only a

single type of fluorescent label, said single type of fluorescent label having the same chemical structure, and said oligonucleotide undergoes a detectable change in fluorescence upon hybridization of said one or more oligonucleotides to said one or more target nucleic acid molecules; and

detecting the presence or absence or quantifying the amount of said one or more target nucleic acid molecules by measuring said fluorescent label.

14. The method of claims 11 or 12, wherein said detection step comprises detecting or measuring the level of activity of the fluorescent label during said synthesis or amplification compared to the level of activity of the fluorescent label in the absence of said synthesis or amplification.

15. The method of claim 12, wherein said amplification is accomplished by at least one method selected from the group consisting of PCR, 5-RACE, RT PCR, Allele-specific PCR, Anchor PCR, "one-sided PCR," LCR, NASBA, and SDA.

17. The method of anyone of claims 11 or 12, wherein said one or more oligonucleotides comprise one or more hairpin structures.

18. A method for amplifying a double stranded nucleic acid molecule, comprising:

providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said nucleic acid

molecule and said second primer is complementary to a sequence within or at or near the 3'-termini of the second strand of said nucleic acid molecule;

hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more polymerases, under conditions such that said primers are extended to result in the synthesis of a third nucleic acid molecule complementary to all or a portion of said first strand and a fourth nucleic acid molecule complementary to all or a portion said second strand;

denaturing said first and third strands, and said second and fourth strands; and repeating the above steps one or more times, wherein one or both of said first and second primers are labeled with only a single type of fluorescent label, said single type of fluorescent label having the same chemical structure;

wherein said primer undergoes a detectable change in fluorescence upon hybridization of said one or more labeled primers to said nucleic acid molecule.

19. The method of claim 18, wherein at least one of said primers comprises at least one hairpin structure.

59. The method of claim 18, wherein said primers further comprise one or more hairpin structures.

63. The method of any one of claims 11, 12, or 18, wherein said detectable label is at the fourth base from the 3' termini.

64. The method of any one of claims 11, 12, or 18, wherein said detectable label is at the fifth base from the 3' termini.

65. The method of any one of claims 11, 12, or 18, wherein said detectable label is at the sixth base from the 3' termini.

66. The method of any one of claims 11, 12, or 18, wherein said detectable label is attached to one of the ten 3'-most terminal nucleotides.

67. The method of any one of claims 11, 12, or 18, ~~or 20~~, wherein said detectable label is attached to one of the twenty 3'-most terminal nucleotides.

78. The method of any one of claims 11, 12 or 18, wherein said fluorescent label is JOE or FAM.

79. The method of any one of claims 11, 12 or 18, wherein said fluorescent label is TAMRA or ROX.

Claims 17 and 63-67 are shown as amended in an Amendment under 37 C.F.R. § 41.33(b), filed concurrently herewith.

IX. Evidence Appendix

Exhibit	Title of Exhibit	Location in Record
Exhibit 1	Horn, U.S. Patent No. 6,465,175	Cited by Examiner in Office Action dated January 4, 2006
Exhibit 2	Tyagi, U.S. Patent No. 6,037,130	Cited by Examiner in Office Action dated January 4, 2006

X. Related Proceedings Appendix

None.